Gastric Cancer and Premalignant Lesions in Atrophic Gastritis: A Controlled Trial on the Effect of Supplementation with Alpha-Tocopherol and Beta-Carotene

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Varis K, Taylor PR, Sipponen P, Samloff IM, Heinonen OP, Albanes D, Härkonen M, Huttunen JK, Laxén F, Virtamo J, The Helsinki Gastritis Study Group. Gastric cancer and premalignant lesions in atrophic gastritis: a controlled trial on the effect of supplementation with alpha-tocopherol and beta-carotene. Scand J Gastroenterol 1998;33:294–300.

Background: Vitamin E and beta-carotene are considered to decrease the risk of gastric cancer both in humans and in laboratory animals. We studied the effect of dietary supplementation with alpha-tocopherol and beta-carotene on the end-of-trial prevalence of premalignant and malignant lesions of the stomach in older men with atrophic gastritis. *Methods:* The study was carried out within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC study) in Finland, in which 29,133 male smokers aged 50–69 years were randomly assigned to receive daily 50 mg alpha-tocopherol, 20 mg beta-carotene, both of these agents, or placebo, for 5–8 years. Serum pepsinogen was determined at base line and after 3 years' supplementation to find men with atrophic gastritis. A low serum pepsinogen I level, indicating atrophic gastritis of the corpus area of the stomach, was found in 2132 men. These men were invited to have upper gastrointestinal endoscopy (gastroscopy), which was performed on 1344 subjects after a median supplementation time of 5.1 years. *Results:* Neoplastic alterations were found in 63 of the men (4.7%): 42 with definite dysplasias of low grade (moderate dysplasia), 7 with definite dysplasias of high grade (severe dysplasia), 11 with carcinomas (of which 7 were 'early' cancers), and 3 with carcinoid tumors. Neither alpha-tocopherol (relative risk, 0.98; 95% confidence interval, 0.57–1.69) nor beta-carotene (relative risk, 1.13; 95% confidence interval, 0.65–1.95) supplementation had any association with end-of-trial prevalence of gastric neoplasias after adjustment for other possible risk factors. The effect was not modified by base-line serum level or dietary intake of vitamins, prevalence of *Helicobacter pylori* infection, or other covariates. *Conclusions:* We thus conclude that supplementation with alpha-tocopherol or beta-carotene for 5 years has no major impact on the occurrence of neoplastic changes of the stomach in older male smokers with atrophic gastritis.

Key words: Alpha-tocopherol; atrophic gastritis; beta-carotene; cancer prevention; gastric cancer; gastric dysplasia; nutrition

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The incidence of gastric cancer has decreased markedly worldwide during the past several decades (1), but this type of cancer is still one of the commonest malignant tumors in much of the world's population. Early diagnosis or preventive measures are possible approaches to improve the outcome for gastric cancer, which at present is usually diagnosed at an advanced stage and therefore has a poor prognosis.

The logical explanation for the decrease in incidence involves changes in lifestyle and dietary factors and a reduction in rate and risk of *Helicobacter pylori* acquisition (2). Epidemiologic studies show that the consumption of large quantities of fruits and vegetables is associated with a reduced risk for gastric cancer (3). A large number of potentially anticarcinogenic substances occur in these foods, including carotenoids and vitamin E, which protect against reactive oxygen metabolites, enhance immune reactions, and inhibit the formation of *N*-nitroso compounds (4). Epidemiologic studies on beta-carotene, vitamin E, and gastric cancer have, however, provided inconsistent results: some studies suggest a protective effect, whereas others show no effect (see Refs. 5, 6).

Atrophic gastritis is a well-known risk condition of gastric cancer (7, 8). Low serum level of pepsinogen I (SPGI) is a good indicator that atrophic gastritis is affecting the corpus area of the stomach. Accordingly, subjects with increased risk

* The participants of The Helsinki Gastritis Study Group are listed in the Appendix.

for gastric cancer can be identified in the general population by screening with the SPGI method (9).

We studied the effect of supplementation with alpha-tocopherol or beta-carotene on the end-of-trial prevalence of gastric neoplastic lesions among men more than 50 years old with atrophic gastritis. For this, we determined SPGI in the participants of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC study) and, after a median supplementation time of 5.1 years, we performed gastroscopy in those with low SPGI.

MATERIALS AND METHODS

This study is an associated study of the ATBC Cancer Prevention Study, which was a randomized, double-blind, placebo-controlled trial to examine the effect of alpha-tocopherol and beta-carotene on the incidence of lung and other cancers (10, 11).

ATBC study

The rationale, design, and methods of the study, the characteristics of the participants, and the measures of compliance have been described in detail elsewhere (10, 11).

The participants of the ATBC Study ($n = 29{,}133$) were recruited from the total male population aged 50 to 69 years living in southwestern Finland ($n = 290{,}406$). To be eligible, each had to have a history of smoking at least five cigarettes per day at entry. A potential participant was excluded if he had a history of cancer or serious disease limiting his ability to participate, was taking supplements of vitamin E, vitamin A, or beta-carotene in excess of defined doses, or was being treated with anticoagulant agents. Each participant was randomly assigned to one of four supplementation regimens: alpha-tocopherol (50 mg/day) only ($n = 7286$), beta carotene (20 mg/day) only ($n = 7282$), alpha-tocopherol and beta carotene ($n = 7278$), or placebo ($n = 7287$). The study agents were formulated as synthetic *dl*-alpha-tocopheryl acetate (50% powder) and synthetic beta carotene (10% water-soluble beadlets). The daily dose was given as a single capsule.

Medical, dietary, smoking, and other background data were obtained at study entry. Dietary intakes of vitamins E and C, beta-carotene, fiber, sodium, and alcohol were estimated from a diet history questionnaire (12) and were available for 27,111 participants (93%).

The recruitment of study participants took place from 1985 to 1988, and the intervention continued until 30 April 1993. The follow-up included three visits per year to the local study center. At each visit each participant returned the capsule pack from the previous period and was given a new pack. Compliance was assessed by counting the capsules remaining at each visit. Overall compliance was excellent, with four of five active participants having taken more than 95% of their capsules. No differences in capsule compliance existed across the intervention groups.

At base line and after 3 years' intervention a blood sample was drawn from each study participant and the serum was stored at −70°C. Serum alpha-tocopherol and beta-carotene concentrations were measured in these serum samples with high-performance liquid chromatography (13). After 3 years of supplementation, serum concentration of alpha-tocopherol had increased by 50% among men receiving alpha-tocopherol, and that of beta-carotene had increased 17-fold compared with the base-line level among those receiving beta-carotene (11).

The ATBC study was approved by the institutional review boards of both the National Public Health Institute, Helsinki, Finland, and the National Cancer Institute, Bethesda, Maryland, USA, and all subjects provided informed consent before entry into the study. A data and safety monitoring committee was convened twice annually throughout the study to review its progress and integrity and to evaluate data relevant to safety and efficacy.

Screening of subjects with atrophic gastritis

The target population of this analysis was men with atrophic gastritis. Their identification was based on low serum pepsinogen levels either in the base-line serum sample or in the sample drawn after 3 years' intervention (midtrial sample).

The study was done in two phases (the pilot and the extended phase). In the pilot phase in 1989–1991 serum pepsinogen I (SPGI) and serum pepsinogen II (SPGII) were determined by one of us (I. M. Samloff) for the ATBC Study participants living in Helsinki and its surroundings (the county of Uusimaa) (14). All men with SPGI <25 μg/l and a ratio of SPGI to SPGII <2.5 were invited to gastroscopy in a study center located in a fully equipped medical center. In the extended phase in 1992–1993 SPGI was determined for the rest of the ATBC Study participants with a modified method using Decanting Solution 3 (Pharmacia, Sweden) (15), and men with an SPGI <49 μg/l (equal to <25 μg/l in the pilot phase assay, the SPGI levels from the extended phase having been transformed to correspond to those from the pilot phase) were invited to gastroscopy in a central or district hospital in their study area.

Of the 8499 men enrolled in the ATBC Study in the Helsinki-Uusimaa area (pilot phase), 6297 men were still active participants in the main study after a median intervention of 3.1 years and underwent serum pepsinogen analyses. Serum pepsinogen levels below the cut-off points were found in 362 base-line serum samples and in 96 midtrial samples among those whose base-line levels of pepsinogens had been above the cut-off level. After a median supplementation of 3.9 years these 458 men were invited to undergo gastroscopy, and 359 (78%) accepted.

Of the 20,634 men enrolled in the ATBC Study outside the Helsinki-Uusimaa area (extended phase), 16,049 men were active participants after a median intervention of 5.4 years, when their SPGI analyses were performed. A low SPGI was

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found in the base-line serum samples of 1393 men and, additionally, in the midtrial serum samples of 281 men. These 1674 men were invited to undergo gastroscopy, and 985 (59%) had gastroscopy after a median supplementation of 5.8 years.

Altogether, of the 2132 men who had had low serum pepsinogen level at study entry or at the midtrial point, gastroscopy was performed in 1344 (63%). The proportion of those not gastroscopied varied non-significantly from 35% to 38% across intervention groups. Reasons for not undergoing gastroscopy were as follows: 152 men had died or dropped out of the main study before invitation to gastroscopy, 127 refused gastroscopy, 146 were excluded because of contraindications (mostly coronary heart disease), and 363 failed to respond to the invitation. Participants and all staff involved in the various phases of the study remained blind to the intervention assignment throughout the trial.

Gastroscopy

Each man with a low serum pepsinogen level was sent a letter explaining the purpose of the study and the risk of neoplastic lesions associated with atrophic gastritis and including an invitation to undergo a gastroscopy. Accepting respondents signed a written informed consent and completed a short questionnaire concerning possible contraindications to gastroscopy. After a review of each man's medical history, outpatient endoscopy was performed in all without contraindications.

Gastroscopy was performed with Olympus endoscopes in the standard manner. Routine biopsy specimens were taken under visual control as follows: one from the distal and one from the proximal antrum along the lesser curvature, two from the middle corpus, and one from the anterior and one from the posterior wall. In addition, multiple biopsy specimens were taken from all endoscopically abnormal lesions (local color changes, ulcers, scars, abnormal folds, polypoid lesions, tumors).

The location of the biopsy specimens was recorded, and specimens were fixed overnight in neutral-buffered formalin and then embedded in paraffin. Histologic sections were stained with hematoxylin–eosin, Alcian blue–periodic acid-Schiff (PAS) (pH 2.5), and modified Giemsa methods.

Histology and diagnosis of malignancy and dysplasia

Gastritis and any related histopathologic appearance in all specimens were classified by means of the Sydney System (16). Dysplasia was diagnosed if epithelial atypia (cellular atypia) occurred in association with abnormalities in differentiation of the epithelium and in achitecture of the gastric foveolae and glands (17–21).

Dysplasia was initially graded into three categories: mild, moderate, or severe with further classification of dysplasias into gastric (foveolar) or metaplastic types (17). The mild category included cases in which the lesions were at most mild in degree, obscure, or indefinite. For instance, all cases with atypic immature metaplasia (intestinal metaplasia type II or III) without other dysplastic features were graded as of this category. Moderate and severe categories included all cases in which definite dysplasia could be established. For instance, all lesions with adenoma-like growth patterns without evidence of malignancy (invasion) were classified as of this category (moderate or severe dysplasia). Cases of carcinoma in situ-type appearance (malignancy without evidence of invasion) were classified as severe dysplasia. Carcinomas (tumors with definite invasion) and carcinoid tumors were noted separately.

In the present investigation gastric carcinomas, carcinoid tumors, and dysplasias of moderate or severe grade were considered to be definitely malignant or premalignant. Cases were accepted as definite dysplasia only when evaluation of the lesions by two independent pathologists (P. Sipponen, Helsinki, and K. Lewin, Los Angeles) were in agreement. Three cases occurred in which opinions diverged, and these were classified as a mild, indefinite category of dysplasia. Within the definite dysplasia category the cases were classified into two subcategories (definite dysplasia, low grade, and definite dysplasia, high grade) after discussion and mutual agreement by the two pathologists.

Safety aspects

This associated gastritis study of the ATBC Study was separately approved by the Ethical Issues Committee of the National Public Health Institute, Helsinki, Finland. A Data and Safety Monitoring Committee dedicated to this study supervised the safety of the gastroscopies.

Electrocardiogram and blood hemoglobin evaluation were done in most of the subjects before gastroscopy. Subjects with symptomatic cardiovascular disease or with major ischemic changes in their electrocardiograms were excluded ($n = 97$).

Four complications occurred in connection with gastroscopy, three of which were self-limiting bleedings after gastric biopsy. The most serious was in a 62-year-old participant who experienced chest pain and dyspnea immediately after a technically trouble-free gastroscopy. He was found to have a dissection of the thoracic aorta, which was successfully operated on within hours. However, because of multiple brain emboli after surgery he was left with permanent weakness in the peripheral musculature. This incident was considered by the Data and Safety Monitoring committee and was seen to be coincidental with rather than a complication of the gastroscopy.

Statistical analysis

Since there is no apparent reason to suspect that the different selection criteria for screening of atrophic gastritis affected the distribution of gastric neoplastic lesions in the four intervention groups, the data of the two study phases were pooled. Logistic regression analysis was used to calculate the odds ratio of premalignant and malignant lesions in atrophic gastritis (22). Moderate (definite dysplasia of low grade) or severe dysplasia (definite dysplasia of high grade),

Table I. Base-line characteristics (median or proportion) among subjects with gastroscopy (n = 1344) in the 4 intervention groups*

Characteristic	AT	AT + BC	BC	Placebo
No. of subjects	321	361	329	333
Age (years)	59.2	59.5	57.9	58.7
Serum pepsinogen I (μg/l)	23.6	21.5	21.5	24.4
Years of smoking	39	39	37	40
Cigarettes/day	20	20	20	20
Alcohol (g/day)	8.5	8.8	7.9	10.5
Dietary fiber (g/day)	25.0	26.4	24.9	24.6
Dietary vitamin E (mg/day)	10.0	10.8	10.3	10.0
Dietary BC (mg/day)	1.50	1.71	1.69	1.63
Dietary vitamin C (g/day)	99	108	103	102
Dietary sodium (g/day)	5.01	5.18	5.01	5.03
Serum AT (mg/l)	11.4	11.8	11.6	11.5
Serum BC (μg/l)	197	194	208	190
Abdominal pain (%)	17.4	12.5	16.1	19.5
Heartburn (%)	14.0	15.2	15.8	11.4
Abdominal pain, heartburn (%)	25.9	22.4	25.8	26.7
H. pylori positivity (histology) (no.)	99	107	97	92
H. pylori positivity (histology) (%)	31	30	29	28

* AT = alpha-tocopherol; BC = beta-carotene.

carcinoma, and carcinoid tumor were combined as end points for this analysis. Mild dysplasia (indefinite dysplasia) was not considered an end-point lesion. Regression models were run with alpha-tocopherol and beta-carotene supplementation groups as explanatory variables with and without adjustment for covariates. Interaction between the supplementation groups and between supplementation and covariates was tested by comparison of nested logistic models with the likelihood ratio test. The covariates included age, smoking years, number of cigarettes daily, dietary fiber, vitamin E, beta-carotene, vitamin C, and sodium, serum alpha-tocopherol, beta-carotene, and SPGI, and alcohol intake at base line, and the phase of performance of gastroscopy (Helsinki-Uusimaa, the pilot phase versus the rest of the ATBC study areas, the extended phase). Continuous variables were divided into medians in the regression models.

RESULTS

Table I shows the characteristics of the gastroscopied subjects at entry to the ATBC Study on the basis of the four intervention groups. No significant differences appeared in any variables between the groups, including the prevalence of *Helicobacter pylori* infection in histology. The median time from the start of supplementation to gastroscopy varied from 5.1 to 5.2 years across the intervention groups. The occurrence of atrophic gastritis in gastric biopsy specimens taken at gastroscopy varied from 79% to 82%.

Among 1344 men with gastroscopies performed, a total of 63 were found to have moderate (definite dysplasia of low grade) or severe dysplasia (definite dysplasia of high grade), carcinoma, or carcinoid tumors. Only 1 of these 63 lesions was found in a random biopsy specimen; all others were found in targeted specimens from abnormal lesions at endoscopy. The cases were evenly distributed across the intervention groups (Fisher's exact test, $P = 0.41$) (Table II), and no significant association of either alpha-tocopherol or beta-carotene supplementation with the end-of-trial prevalence of gastric neoplasias was observed in logistic regression analysis with or without adjustment for covariates (Table III). Nor was there any evidence of interaction between the two supplements (alpha-tocopherol, beta-carotene) in their effect on neoplasias (likelihood ratio test, $P = 0.25$). Furthermore, no interactions were found between the supplements and the covariates.

Of the confounding variables, only duration of smoking years was associated with risk of gastric neoplasias. In the regression model the relative risk of gastric neoplasias among men above the median for smoking years (at least 39 years) was 2.34 (95% confidence interval, 1.18–4.65) compared with

Table II. End-of-trial number of patients with any definite dysplastic or neoplastic lesion in the four intervention groups

Type of lesion	AT*, n = 321	AT + BC*, n = 361	BC*, n = 329	Placebo, n = 333	Total, n = 1344
Definite dysplasia, low grade	8	13	6	15	42
Definite dysplasia, high grade	2	2	3	0	7
Carcinoma	2	4	3	2	11
Carcinoid tumor	1	0	1	1	3
Total	13	19	13	18	63

* AT = alpha-tocopherol; BC = beta-carotene.

Table III. Association of supplementation with alpha-tocopherol (AT) or beta-carotene (BC) with end-of-trial prevalence of gastric mucosal neoplasia–dysplasia lesion in logistic regression model (relative risk and its 95% confidence intervals)

Model	AT versus no AT	BC versus no BC
Unadjusted RR	1.00 (0.60–1.66)	0.98 (0.59–1.62)
Adjusted RR*	0.98 (0.57–1.69)	1.13 (0.65–1.95)

*Adjusted for base-line age, smoking years, cigarettes smoked per day, alcohol consumption, dietary fiber, dietary vitamin E, dietary beta-carotene, dietary vitamin C, dietary sodium, serum alpha-tocopherol, serum beta-carotene, and serum pepsinogen I as medians, as well as geographic area from which subjects were selected: Helsinki with surrounding Uusimaa county versus other areas.

men below the median. The number of cigarettes smoked per day did not, however, show any significant effect.

DISCUSSION

In the present study we found no effect of alpha-tocopherol or beta-carotene on gastric carcinomas or definite premalignant lesions (dysplasia) after a median supplementation of 5 years. To ascertain the end-of-trial carcinomas and dysplasias, we endoscoped those who were at high risk for gastric cancer—that is, had low SPGI. On endoscopy of 1344 men with low SPGI, we found 63 who had carcinoma or dysplasia. This number of cancer–dysplasia cases was small, however, and led to quite wide 95% confidence intervals for relative risks, which include even the possibility of a 40% decrease in risk.

In the parent ATBC study itself, from which our 1344 men with gastroscopy were drawn, 126 incident cases of stomach cancer (in the great majority of the cases, advanced) were diagnosed during the study period among the 29,133 participants (10). Neither alpha-tocopherol nor beta-carotene supplementation had a preventive effect in these cases. Thus, data from combining the parent and the present study make it seem unlikely that supplementation for 5 years with alpha-tocopherol or beta-carotene at the doses used in the present study has any major effect on the rate of gastric cancer in older Finnish male smokers.

The present findings contradict epidemiologic and animal studies suggesting that alpha-tocopherol and beta-carotene inhibit gastric carcinogenesis (3–6). In an intervention study on gastric cancer in Linxian, China, in the late 1980s, a favorable result was obtained with regard to cancer prevention by vitamin supplementation (23). In this trial with 29,000 participants, a reduced incidence of (relative risk, 0.84; 95% confidence interval, 0.71–1.00) and mortality from gastric cancer (relative risk, 0.79; 95% confidence interval, 0.64–0.99) were found among those who for 5 years received daily supplementation with a combination of beta-carotene (15 mg), vitamin E (30 mg), and selenium (50 μg). The target population of the Linxian study differed greatly from that of the ATBC study, however, particularly with regard to diet and base-line levels of serum vitamins and micronutrients. In the ATBC study very few participants had an inadequate vitamin intake, but such inadequacy was common in Linxian. For example, only 3% of the ATBC participants had serum levels of alpha-tocopherol below 7 mg/l, whereas the corresponding figure in Linxian was about 50% (24). It is, therefore, possible that the positive results of the Linxian study are due to correction of existing vitamin deficiences rather than to supplementation above nutritionally adequate intake levels.

In another 6-year intervention study from Linxian (25), in which 3000 persons with esophageal dysplasia received daily a combination of 14 vitamins and 12 minerals, including beta-carotene (15 mg) and vitamin E (60 IU), no significant differences were found in the incidence of (relative risk, 1.17; 95% confidence interval, 0.87–1.58) or mortality from gastric cancer (relative risk, 1.18; 95% confidence interval, 0.76–1.85) between the supplementation and control groups.

In the present study we focused on subjects in whom the pathogenesis of gastric carcinoma was based on atrophic corpus gastritis. A low serum level of PGI and a low PGI to PGII ratio are good markers for the atrophic gastritis affecting the corpus and fundus of the stomach (9). In keeping with this, we found moderate or severe atrophic gastritis in the corpus biopsy specimens in 81% of the subjects who had had endoscopy, a rate ranging from 79% to 82% across all intervention groups.

It is realistic to assume some degree of misinterpretation of mucosal lesion during endoscopy and microscopy, although there is no reason to believe that this bias would be dissimilarly associated with the intervention groups. Misclassification of the end point, however, leads to some degree of underestimation of supplementation effect. To avoid this misclassification, the present study was based on clear lesions: cancer and definite dysplasia. Both the endoscopy and the microscopic evaluation of biopsy specimens were done blind and before breaking the study code. In addition, the histologic evaluation of dysplastic and malignant lesions was performed by two experienced pathologists independently, to ensure that only malignant or definitely precancerous lesions were recorded as end-point lesions.

For various reasons 37% of the men with low serum PGI did not undergo gastroscopy. These and the dropouts from the parent study before serum pepsinogen determination could cause bias in the findings. However, the withdrawals from the gastroscopy and the dropouts from the parent study were equally distributed across the intervention groups. In addition, base-line levels of various background variables, including the occurrence of *H. pylori* infection, were equally distributed across the intervention groups among the subjects with gastroscopy, and, furthermore, these variables were adjusted for in the logistic regression model in evaluation of risk of gastric neoplasia.

The duration of our supplementation before gastroscopy, a median of 5.1 years, may have been too short to cause any noticeable change in the risk of gastric carcinoma or of

precancerous lesions. Gastric cancers, which arise in a metaplastic, atrophic gastric mucosa, obviously result from a very long process. Atrophic gastritis itself is a common disease that may last decades (26). Because the risk of gastric cancer increases along with an increase in grade and extent of atrophic gastritis (9) it is difficult to estimate the average time interval from the early appearance of atrophic gastritis to the point at which definitely premalignant or malignant lesions appear. This interval may vary but is obviously many years or, possibly, decades. If the neoplastic lesions progress slowly, some of them may have already been in existence at the start of the intervention, attenuating the possible chemopreventive effect.

An interesting observation from the present study was that a long smoking history appeared to be a significant risk factor for gastric cancer. Accordingly, a smoking habit of more than 39 years may be an even stronger risk factor for gastric cancer than diet, at least in a well-nourished population. The number of cigarettes consumed per day did not, however, show any association with gastric cancer or with premalignant gastric lesions. Other studies have shown an increased risk of gastric cancer among smokers, although most studies have failed to show a clear dose-response relation (27). In accordance with the present finding, a Swedish case–control study (28) also showed a positive association between the duration of smoking and gastric cancer risk.

To conclude, we found that dietary supplementations with alpha-tocopherol or beta-carotene for 5 years had no major effect on the end-of-trial prevalence of premalignant and malignant gastric mucosal alterations in subjects with baseline atrophic gastritis. Our results do not, however, exclude the possibility that supplementation with alpha-tocopherol and beta-carotene may have some protective effect against gastric cancer in some populations, such as in the general population of Linxian, China, where inhabitants are commonly deficient in several micronutrients.

Vitamins A, E, C, and their analogues have been shown to play a role in experimental carcinogenesis of many tumors, either by promoting or by enhancing the tumor formation (29). These compounds prevent free radical reactions and inhibit nitrosation, alpha-tocopherol showing this action in, for instance, lipid compartments of tissues, similarly to the action of vitamin C in aqueous solutions (29). Vitamins may also interact with minerals and other non-nutrient dietary components. This may indicate that the influence of vitamin supplementation in carcinogenesis is complex in populations with good nutrition and high or adequate intake of vitamins and minerals in particular.

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APPENDIX

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